The protein C pathway, oral contraceptives and venous thrombosis

Chapter 8

General summary and conclusions

Venous thrombosis is recognized to be a multicausal disease. In recent years more and more genetic and acquired risk factors have been identified that play a role in the development of venous thrombosis. One major risk factor that was discovered in 1993 is activated protein C (APC) resistance. Hereditary APC resistance results from a single point mutation in coagulation factor V. The mutated molecule, often called FV_{Leiden}, lacks an APC cleavage site and therefore, its procoagulant activity cannot be efficiently down-regulated by APC. As a consequence, the plasma of carriers of the FV_{Leiden} mutation is APC resistant. However, 10% of the APC resistant individuals are not carrying the FV_{Leiden} mutation and hence have a different, as yet unknown cause for APC resistance[1]. Moreover, APC resistance without the FV_{Leiden} mutation has also been established as a risk factor for venous thrombosis[2]. Theoretically, APC resistance not caused by FV-Leiden could be induced by altered levels in haemostatic factors that enhance thrombin generation or that hamper the action of the anticoagulant protein C pathway, i.e. 1) high concentrations of procoagulant proteins (*e.g.* prothrombin, factor VIII), 2) low concentrations of inhibitors of coagulation (*e.g.* tissue factor pathway inhibitor), 3) low concentrations of anticoagulant proteins (*e.g.* protein S) or 4) high concentrations of inhibitors of anticoagulant proteins (*e.g.* protein C inhibitor). It is also possible that combinations of these occur and act in concert to render an individual's plasma APC resistant.

Rather than trying to identify single contributing risk factors by a combination of individual measurements, it is useful to assess a possible prothrombotic condition via a single overall assay. The APC resistance test used throughout the investigations described in this thesis, is such a measurement. This assay quantifies the effect of APC on thrombin generation initiated in plasma via the extrinsic pathway. The first results obtained with this test, revealed that women using oral contraceptives (OC) were more resistant to APC than women not using OC. Moreover, users of so-called third generation OC exhibited responses to APC equal to that of individuals with the FV_{Leiden} mutation[3]. Since the observed APCsr's correlated with earlier reported risks of venous thrombosis for carriers of FV-Leiden and of users of different types of OC (i.e. second and third generation), it was proposed that acquired APC resistance could provide a biological explanation for the increased risk of thrombosis associated with the use of OC.

In Chapters 2, 3 and 4 of this thesis the use of this assay for the assessment of APC resistance is described. In Chapters 5, 6 and 7, the effects of changes of female sex hormones on the anticoagulant pathway are reported. In this discussion a summary will be given of the different investigations described in this thesis.

Characteristics of the APC resistance test

In Chapter 2 we have described the effects of analytical and pre-analytical variables on APCsr determined with our APC resistance test. The effects of variation of the concentration of the different reactants used in the test (calcium, phospholipids, tissue factor and APC) on thrombin formation were determined. We found that APC inhibits by thrombin formation in a dose-dependent manner. The inhibition by APC was affected by the concentration of tissue factor, calcium and phospholipids. In order to obtain reproducible APCsr, the concentrations of these reactants should therefore, be standardized. We have chosen the conditions of this assay such, that normal pooled plasma has a residual thrombin formation in the presence of APC of approximately 10%. Small variations in residual thrombin formation, due to variations of reactants, will influence the outcome of the assay in both normal and patient plasma. This may result in a day-to-day variation that can be minimized by normalisation i.e. by dividing the APCsr of a particular plasma sample by the APCsr obtained in normal pooled plasma[4].

The influence of different procedures to process whole blood to plasma, was also investigated. These included 1) concentration of citrate used to anticoagulate whole blood, 2) time before processing, 3) time and speed of centrifugation, 4) storage temperature of plasma and 5) an additional thawing of a frozen plasma sample. Differences in centrifugation and additional thawing of plasma had no effect on the APCsr, whereas a minor effect was observed for the time waited before sample processing as well as for the storage temperature. However, variation in the concentration of sodium citrate, used to anticoagulate whole blood upon drawing, has considerable influence on the APCsr. This is likely due to the fact that the anticoagulant action of APC is critically dependent on the calcium concentration. In different laboratories the sodium citrate concentration that is used to anticoagulate whole blood varies between 0.105 and 0.129M. Thus, the citrate concentration appears to influence the APCsr, an effect that has also been reported for APCsr

values determined with clotting times in the aPTT[5] . To at least partially reduce citrate effects, it is recommended to normalize APCsr against a pooled normal plasma that is collected on the same concentration of anticoagulant.

Hereditary and acquired APC resistance

The APC resistance assay described in this thesis is sensitive to both hereditary and acquired risk factors for venous thrombosis (Chapter 3). Carriers of the prothrombin mutation (PT G20210A), as well as individuals with protein S deficiency had higher APCsr than controls. APC resistance in carriers of the prothrombin mutation, which is associated with high levels of prothrombin, may be explained by an inhibition of APC-dependent factor Va inactivation[6]. Moreover, high levels of prothrombin might increase the amount of thrombin formed, leading to excessive formation factor Va. This poses an increasing demand on APC to down-regulate thrombin formation, which may lead to an APC resistant state of plasma.

Protein S is a cofactor of APC in the inactivation of factor Va. Hence, at low levels of protein S down-regulation of thrombin formation is impaired, which results in APC resistance. Protein S deficiency can be both of hereditary and acquired origin. Hereditary protein S deficiency can be caused by mutations in the protein S molecule. Acquired protein S deficiency, on the other hand, is associated with the use of hormone preparations (OC), and with pregnancy.

In chapter 3 it is also described that co-existence of risk factors for venous thrombosis that increase the APCsr, cause an even higher APCsr and in some cases render the plasma of affected individual almost completely resistant to the action of APC. The finding that more than one risk factor needs to be present to eventually cause thrombosis, is thus reflected in the APCsr values. This idea is supported in Fig3.5, which shows that there is a good correlation between the APCsr of individuals with hereditary and/or acquired risk factors for venous thrombosis and the reported odds ratios.

Clotting or thrombin formation

In chapter 3 it is shown that acquired APC resistance is especially observed in women who are using OC or who are pregnant. Depending on the APC resistance test used, there appears to be a variable number of individuals who are acquired

APC resistant. The molecular basis of this acquired APC resistance is, however, unclear. With the intention to gain more insight in this, we have assessed the sensitivity to APC of FV_{Leiden} carriers and OC users with two different APC resistance assays (Chapter 4): 1) the thrombin generation assay, which is very sensitive to hormonal influences (e.g. OC, HRT and pregnancy) and thus to acquired APC resistance and with 2) an APC resistance assay that is routinely used in hospital laboratories and that evaluates the effect of APC on the clotting times of plasma in which coagulation is initiated via the intrinsic pathway (*i.e.* the activated partial thromboplastin time, aPTT).

Both assays were equally sensitive and specific in determining the FV_{Leiden} mutation. However, the increased APC resistance during OC use is hardly observed in the classical clotting assay. Thus, apart from the ability to detect the FV_{Leiden} mutation, the correlation between the two assays is rather poor. There are at least two possible explanations for these observed differences:

1) In the two assays coagulation is initiated with activators which activate different pathways of coagulation (tissue factor versus kaolin). Tissue factor-induced coagulation mainly proceeds via the extrinsic pathway, whereas kaolin activates the intrinsic pathway. Plasma proteins of each pathway may be differentially modulated during OC use and this can lead to different sensitivities of the plasma for APC. For example, the APCsr measured in the aPTT is influenced by high levels of factor VIII, whereas the APCsr determined in the ETP-based test is sensitive to changes in protein S.

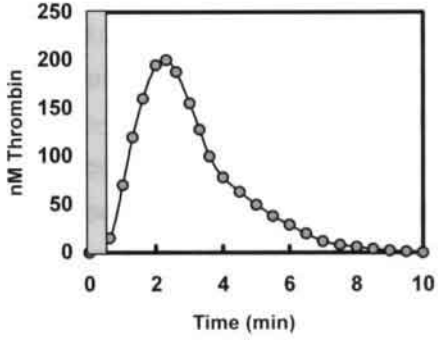

Figure 8.1 Already after formation of the first traces of thrombin, clotting is achieved, as indicated by the grey bar. However, measurement of thrombin generation represents the total amount of thrombinthat has been formed during coagulation.

2) The endpoint determination of the two assays is different *i.e.* clotting time versus thrombin formation. Clotting occurs already after the first traces of thrombin are formed (i.e. in approximately 30 sec), whereas the measurement of the endogenous thrombin potential reflects the total amount of thrombin that has been formed over a prolonged time period (Fig 8.1). It can be hypothesized that the first phase of coagulation (*i.e.* when the first traces of thrombin are generated and the clot is formed) is less sensitive to modulation by (anti)coagulation factors than the endogenous thrombin potential which quantifies the amount of thrombin that has been formed over a much longer time period. However, further experiments are needed to test these hypotheses.

APC resistance during the use of oral contraceptives

In PART II of this thesis the effects of changes in female sex steroid hormones on APC resistance protein C system are described. From the very early beginning on, the use of oral contraceptives has been associated with an increased risk for venous thrombosis. However a satisfying biological explanation for the observed increased thrombotic risk was lacking, until 1997 when acquired APC resistance [3] was proposed as a possible mechanism to explain the occurrence of venous thromboembolism in OC-using women.The study published in 1997 concerned a cross-sectional, non-cycle controlled design the results of which could have been influenced by uncontrolled pill effects and selection bias. Therefore, a double blind randomized cross-over trial of two oral contraceptive preparations was performed. The cross-over study showed that a large number of haemostatic variables changed during the use of OC (procoagulant, anticoagulant and fibrinolytic). The changes of most of the coagulation factors were more pronounced during the use of desogestrel-containing OC than during the use of levonorgestrel-containing OC. Notably, more pronounced APC resistance was observed during the use of the desogestrel-containing preparation than on levonorgestrel-containing OC.

Differences between the effects of second and third generation pills on the level of anticoagulant proteins were only found for protein S. Decreases in protein S were more pronounced during the use of third generation OC. Moreover, changes of total and free protein S correlated negatively with the increase of the APCsr determined with the ETP-based APC resistance assay (r= -0.44 / -0.60), which

indicates that the decrease in protein S can, at least partially, explain increases in APCsr. However, other mechanisms leading to acquired APC resistance must also be involved, since protein S did not significantly change during the use of second generation OC, whereas the APCsr increased considerably. Particularly, the combination of higher levels of factor VII, X and prothrombin[7] will result in an increased procoagulant pressure that may further contribute to the APC resistant phenotype found in OC using women.

Considering the discussion about OC use and the risk of venous thrombosis, it was proposed that the increased thrombin formation and decreased anticoagulant action could partially be counteracted by an increased fibrinolysis. The cross-over study confirmed that levels of fibrinolytic proteins increase during the use of OC, however, no shortened clot lysis time was found in these samples. Meijers et al.[8] report a progestagen specific difference for TAFI (thrombin activatable fibrinolysis inhibitor). TAFI was increased during the use of OC, especially during third generation OC. The increase in TAFI together with the elevated thrombin formation, probably yields more activated TAFI that can counterbalance the effects of increased levels of fibrinolytic proteins by inhibiting fibrinolysis.

Acquired APC resistance during HRT, pregnancy and IVF

The synthetic hormones in oral contraceptives cause changes in procoagulant (e.g. prothrombin, factor VII and factor X), anticoagulant (protein S total and free, Chapter 5) and fibrinolytic proteins (TAFI). Pregnancy, during which endogenous estradiol and progesterone are substantially increased, also induces major changes in the haemostatic system (factor VIII, protein S and antithrombin). Moreover, pregnancy, the use of OC and hormone replacement therapy lead to acquired protein C resistance (Chapter 3). Although, the hormones administered during OC therapy differ from the naturally occurring hormones (such as during pregnancy) the effects on haemostatic parameters are similar.

In chapter 6 the effect of changes in endogenous estradiol en progesterone on the protein C pathway are described. In plasma of women who followed an in vitro fertilisation (IVF) protocol (n=31) and subsequently became pregnant (n=6) the levels of hormones and anticoagulant proteins were determined. During IVF the levels of endogenous estradiol and progesterone increase within a short period of time.

During IVF treatment, no changes in protein S, protein C and protein C inhibitor, but an increase in APC resistance was observed. The estradiol levels correlated well with the APC sensitivity ratios. Moreover, the increase in APC resistance showed a significant correlation with the increase in 17ß-estradiol (r=0.7, p=0.001). In this study six women became pregnant following IVF. In these pregnant women a rapid increase in estradiol and progesterone was observed, concurrent with a rise in APCsr and a decrease in protein S. Although hormone levels during early pregnancy were similar to those during hyperstimulation (E2) and luteal support (P), the effects on APCsr and protein S were more pronounced. This might be caused by an up-regulation of hormone-receptors already early in pregnancy.

Despite considerable changes in estradiol and progesterone, the levels of hemostatic factors and the APCsr changed only minimally and less than observed during OC use. A possible explanation for this might be that synthetic hormones exhibit higher affinity for and have more pronounced effects on hormone receptors than natural hormones[9, 10].

Progesterone may counterbalance the prothrombotic effects of estrogen

Since lowering of the estrogen content in (second and third generation) OC led to an overall decrease in the risk for VTE as compared to first generation preparations, estrogen was held responsible for thrombosis associated with hormonal changes in women. However, since second and third generation OC preparations contain the same amount of ethinyl estradiol (30µg) this suggests that progestagens are, at least partially, responsible for the increased risk of VTE during use of third generation OC. Therefore we have compared the effects of desogestrel and levonorgestrel on the protein C pathway during the use of combined OC, containing ethinyl estradiol (EE) and progestagen, and progestagen-only preparations in women with and without the FV_{Leiden} mutation (Chapter 7). In both populations desogestrel-containing (DSG) combined preparations had more pronounced effects on the anticoagulant pathway than levonorgestrel-containing (LEV) OC. Effects of desogestrel-containing combined OC were more pronounced in carriers than in non-carriers of the FV_{Leiden} mutation. During the use of progestagen-only preparations reverse effects on anticoagulant proteins were found, which in general were more pronounced for levonorgestrel than for desogestrel. The findings presented in this

chapter can explain the increased risk for desogestrel users, especially in the case of carriership of the FV_{Leiden} mutation combined with OC use[11].

In a small study it was proposed that progestagens had a "compensating" effect on coagulation, since APC sensitivity ratios declined with increasing progestagen content in triphasic OC[12]. If progestagens indeed compensate the action of estrogens on hemostasis, it is appealing to reduce the level of ethinyl estradiol in oral contraceptives (so called sub-30 pills) and at the same time maintain or even increase the level of progestagen. The results described in this thesis relate to two different kinds of OC preparations, both containing 150µg progestagen (either levonorgestrel or desogestrel). It is interesting to investigate the influence on hemostasis of OC containing norgestimate, a progestagen that is third generation, but which is metabolised to levonorgestrel. Furthermore, it would be interesting to investigate the changes in haemostasis during the use of OC that contain the same amount of progestagen in combination with a lower EE content e.g. Mercilon® (20µg EE and 150µg DSG). However, also the newest sub-30 pills that contain gestodene (Meliane®, Minulet®) should be compared to their older counterparts that contain more EE and the same amount of gestodene (Femodeen® and Harmonet®, respectively).

In 1997 the Committee for Proprietary Medicinal Products (CPMP) of the European Medicines Evaluation Agency (EMEA) requested the oral contraceptive manufacturers to perform a study to evaluate the effects of seven monophasic oral contraceptives on the haemostatic system. In this study the effect of seven different combination preparations on a large number of haemostatic variables have been investigated[13]. Also a comparison of the effect of different progestagens (gestodene, DSG, LEV and NGM) and different concentrations of EE (20 vs 30 vs 50 µg EE) was made in this study. Although there are indications that this study has been completed, the detailed results of this investigation have as yet not been published.

Can changes of haemostatic parameters explain pill thrombosis?

We have shown in all our studies (cross-sectional, cross-over and longitudinal designs) that the use of combined oral contraceptives is associated with a decreased anticoagulant potency of the protein C pathway as reflected by an increased APC resistance. Moreover, the effects of oral contraceptives containing desogestrel are

more pronounced than levonorgestrel-containing OC. Our studies and other reports have indicated that the changes during OC use in levels of factor VII, factor V, prothrombin and protein S represent an important modulatory effect that in part can explain the observed difference in risk of venous thrombosis between users of second and third generation OC use. Our studies further indicate that individuals with FV_{Leiden} (who already have impaired APC-dependent down regulation of coagulation) become even more resistant to APC during OC use (especially when they use desogestrel-containing OC), and this can explain the high risk for venous thrombosis in carriers of the FV_{Leiden} mutation who use OC.

Since changes in haemostatic variables during OC use are relatively small and the plasma levels of coagulation factors in most women stay within normal ranges, the relevance of changes during OC use has been questioned. For a number of reasons we would like to challenge this interpretation.

Recent reports have indicated that elevated levels of prothrombin[14], factor VIII[15, 16], factor IX[17] factor XI[18] and TAFI[19] are independent risk factors for venous thrombosis. Even when the changes in levels of these proteins are relatively small, the risk of VTE is significantly increased. The effects of OC on the coagulation system are generally judged on the basis of mean changes of haemostatic parameters relative to the mean level of that parameter in the population. However, it is important to realise that, although a change in population average can stay within the normal range, such changes result in an increased number of individuals at the extreme of the normal population distribution.

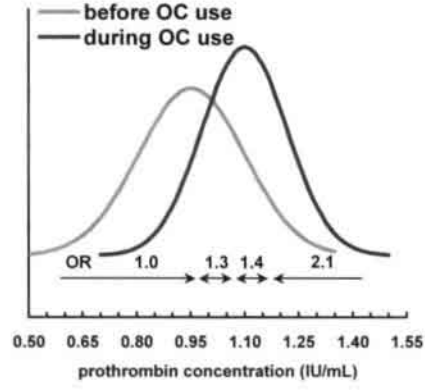

Figure 8.2 Normal distribution of the level of prothrombin before and during the use of OC. Odds ratio's of venous thrombosis reported for the level of prothrombin[12] are indicated in the figure along the x-axis.

Fig 8.2 illustrates this for the level of prothrombin before and during the use of OC. It has been shown that individuals with prothrombin levels >1.15 IU/mL have a 2.1-fold increased risk for developing VTE[14]. The normal distribution of levels of prothrombin, i.e. without OC, are indicated in light-gray. Of the population only 12% has a prothrombin level above 1.15 IU/mL. However, during the use of OC this curve is shifted to the right and the number of individuals exposed to such high levels is increased substantially to 42%. It is unfortunate that in the discussion with regard to the effects of OC use, this phenomenon, which will lead to an increased number of women at risk, is underscored.

In most women the changes in hemostatic parameters during OC use will result in a moderate disbalance, however, the few individuals that already have a mild prothrombotic condition before they use OC (*e.g.* elevated levels of factor II, VIII, IX, XI or low levels of protein S, factor V), may be put in extreme danger during the use of OC, because the concentration of coagulation factors will further change during use. In these women OC may further impair the coagulation system and aggrevate the prothrombotic condition, a situation that in a small number of pill users (7/10000 women years) may culminate in the development of venous thrombosis.

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Nederlandse Samenvatting

Veneuze trombose is een ziekte die door meerdere faktoren wordt veroorzaakt. In de laatste jaren is een groot aantal genetische en verworven risicofaktoren, die een rol spelen bij het ontstaan van veneuze trombose, geïdentificeerd. Een van de belangrijkste risicofaktoren voor veneuze trombose is ontdekt in 1993 en heet APC resistentie. Geaktiveerd protein C (APC) is een enzyme dat ervoor zorgt, dat stolling van bloed tijdig geremd wordt, zodat er geen trombose optreedt. Maar in een groot aantal patiënten met veneuze trombose blijkt dat APC minder goed te werken (het plasma is APC resistent). APC resistentie kan op twee manieren worden veroorzaakt: door een genetische afwijking (erfelijk) en/of door omgevingsfaktoren die tijdelijk aanwezig zijn (verworven). Erfelijke APC resistentie is het gevolg van een puntmutatie in stolfaktor V. Het gemuteerde molekuul, FV_{Leiden} genaamd, mist een belangrijke knipplaats voor APC, waardoor de stollingsbevorderende werking van FV niet goed gereguleerd kan worden. Plasma van FV_{Leiden}-dragers is dus minder gevoelig voor APC (APC resistent). De oorzaken van verworven APC resistentie zijn minder eenduidig. Theoretisch kan verworven APC resistentie het gevolg zijn van een verhoogde neiging tot trombinevorming veroorzaakt door een verandering in niveau van een of meerdere stolfaktoren. Dit kan een gevolg zijn van 1) een verhoogde concentratie van stollingsbevorderende eiwitten (bijv. protrombine of faktor VII), 2) een verlaagde concentratie van remmers van de stolling (bijv. tissue faktor pathway inhibitor), 3) lage concentratie van antistollingseiwitten (bijv. protein S) en/of 4) een hoge concentratie van remmers van het antistollingssysteem (bijv. protein C inhibitor).

Het is daarom niet eenvoudig aan te geven welke hemostase testen de voorkeur verdienen voor het diagnostiseren van verworven APC resistentie. Eén algemene test, waarvan het resultaat een samengestelde meting van pro- en anticoagulante effecten (een "protrombotische toestand") weergeeft, zou daarom heel nuttig zijn. De in dit proefschrift beschreven APC resistentie test kan in een aantal gevallen als zo'n algemene test beschouwd worden. Deze test kwantificeert het effect van APC op de totale trombinevorming na aktivatie van bloedplasma via het extrinsieke stolsysteem.

In Deel I van het proefschrift zijn achtereenvolgens beschreven: de experimentele omstandigheden waaronder de in Maastricht ontwikkelde APC resistentiemeting dient te worden uitgevoerd (hoofdstuk 2); voor welke veneuze

trombose risicofaktoren (erfelijk danwel verworven) de meting gevoelig is (hoofdstuk 3); alsmede een vergelijking van de resultaten met de meer algemeen in gebruik zijnde methode, waarin APC resistentie bepaald wordt door meting van het effect van APC op de stoltijd (hoofdstuk 4). In deel II (hoofdstukken 5-7) wordt vervolgens nader ingegaan op de effecten van veranderingen in de vrouwelijke hormoon huishouding op stollingsparameters, die de werking van het zgn. "protein C pathway" bepalen.

Deel I Eigenschappen van de APC resistentie-test

In hoofdstuk 2 wordt de invloed beschreven van variatie in de concentratie van de verschillende "ingrediënten" van de test (calcium, fosfolipiden, tissue faktor en APC) op de trombinevorming. De remming van de trombinevorming door APC blijkt afhankelijk te zijn van de concentratie tissue faktor, calcium en fosfolipiden. Om reproduceerbare getallen te verkrijgen, moeten de concentraties van deze reactanten daarom worden gestandaardiseerd. De dag tot dag variatie die desondanks aanwezig blijft, kan worden geminimaliseerd door de APCsr (APC sensitivity ratio) van een bepaald plasmamonster te delen door de APCsr van normaal plasma. Het effect van variaties in plasmabereiding uit volbloed is ook bepaald. De geteste variabelen zijn 1) de concentratie van het antistollingsmiddel citraat, 2) de tijd voordat begonnen wordt met de opwerking; 3) centrifugatietijd en -temperatuur gebruikt bij verwijdering van de cellulaire fraktie uit het bloedplasma; 4) opslagtemperatuur van het plasmamonster en 5) een extra keer ontdooien alvorens te meten. Het blijkt dat eigenlijk alleen de concentratie van citraat de APCsr waarden beïnvloedt. Om een goede vergelijking te waarborgen is het dan ook van belang dat de APC resistentie meting uitgevoerd wordt met plasmamonsters die in gelijke concentratie citraat afgenomen zijn.

De resultaten in hoofdstuk 3 maken duidelijk dat de APC resistentietest, zoals beschreven in dit proefschrift, niet alleen gevoelig is voor de faktor V_{Leiden} mutatie, maar ook voor andere risicofaktoren voor veneuze trombose. Dit kunnen zowel erfelijke (de protrombine G20210A mutatie; protein S deficientie) als verworven (zwangerschap, pilgebruik, hormoonsubstitutie therapie) risicofaktoren zijn. Tevens blijken de effecten van de risicofaktoren op APC resistentie in veel gevallen additief te zijn, zodat bij het gelijktijdig voorkomen van risicofaktoren extra

hoge APCsr waarden gevonden worden. Het feit dat de gemeten APCsr waarden correleren met in de literatuur gerapporteerde risico's van trombose suggereert dat de door ons ontwikkelde meting klinisch relevante uitkomsten oplevert.

In het laatste hoofdstuk van deel I (hoofdstuk 4) zijn de resultaten vergeleken met een meer algemeen in gebruik zijnde APC resistentie test (de zgn. aPTT), waarin het effect van APC gemeten wordt op basis van stoltijdmetingen in plasma geïnitieerd via de intrinsieke weg . Beide testen zijn even specifiek en gevoelig voor de aanwezigheid van de FV_{Leiden} mutatie, maar afgezien hiervan blijken de uitkomsten van beide testen verder nauwelijks gecorreleerd. Dit wijst erop dat beide APC resistentie meting via deze testen door verschillende faktoren beïnvloedt word en dat deze verschillende effecten teweeg brengen in de beide APC resistentie testen.

Deel II Hormonale beïnvloeding van APC resistentie

Reeds vanaf de beginjaren '60 is het gebruik van orale contraceptie geassocieerd met een verhoogd risico op veneuze trombose. Echter, totdat in 1997 APC resistentie als mogelijk mechanisme werd voorgesteld, ontbrak een biologische verklaring. In dat jaar werd namelijk gerapporteerd, dat de -in Maastricht ontwikkelde- test liet zien dat pilgebruik niet alleen tot APC resistentie leidt maar ook dat het gebruik van de zgn. derde generatie (bv desogestrel bevattende) pil tot een duidelijk hogere resistentie leidt dan het gebruik van tweede generatie (levonorgestrel bevattende) preparaten. Aangezien deze eerste resultaten sterk bekritiseerd werden is een tweede studie opgezet waarin het pileffect gemeten werd in een dubbel-geblindeerd gerandomiseerd cross-over experiment met twee combinatiepreparaten (hoofdstuk 5). Deze studie liet zien dat een groot aantal hemostase variabelen veranderen tijdens pilgebruik (stollingsbevorderende, antistollende en fibrinolytische eiwitten). De veranderingen waren in het algemeen sterker tijdens het gebruik van desogestrel bevattende pillen dan tijdens het gebruik van contraceptiva met levonorgestrel. Tevens werd aangetoond dat de verworven APC resistentie meer uitgesproken was bij het gebruik van desogestrel-bevattende (derde generatie) preparaten dan bij de levonorgestrel-bevattende preparaten. Verschillen in de effecten van de twee pilpreparaten op de antistollende eiwitten werden alleen

gevonden voor protein S. Protein S niveaus daalden sterker tijdens desogestrel gebruik. Bovendien correleerde de daling in zowel totaal als vrij protein S negatief met de stijging in APCsr waarde (r= -0.44 / -0.60). Dit wijst erop, dat de stijging in APC resistentie voor een deel verklaard kan worden door de daling in protein S. Maar andere mechanismen moeten ook bijdragen aan de gestegen APC resistentie, want tijdens het gebruik van de levonorgestrel bevattende pil veranderde de protein S concentratie nauwelijks terwijl de APCsr significant steeg.

Niet alleen synthetische hormonen (zoals in de anticonceptiepil) zijn in staat het hemostatische evenwicht te veranderen. Ook zwangerschap, wat gepaard gaat met een forse toename van de endogene estradiol- en progesteronspiegels, leidt tot significante veranderingen in stollingsparameters(zie ook hoofdstuk 3). In hoofdstuk 6 zijn de veranderingen in het protein C systeem als gevolg van de veranderingen in endogeen estradiol en progesteron beschreven. De concentratie van hormonen en stollingseiwitten werden bepaald in plasma van vrouwen die een in vitro fertilisatie (IVF) protocol volgden (n=31) en vervolgens zwanger raakten (n=6). Tijdens een IVF-cyclus veranderen de spiegels van endogeen estradiol en progesteron aanzienlijk in een kort tijdsbestek. In deze studie vonden we geen of weinig verandering in protein S, protein C en protein C inhibitor, maar wel een (lichte) verhoging van de APC resistentie. Estradiol spiegels bleken te correleren met APCsr waarden en bovendien was de verandering in 17β-estradiol gecorreleerd met de verandering in APC resistentie. Zes vrouwen werden zwanger na de IVF behandeling. In deze vrouwen werden hoge estradiol en progesteron waarden gevonden gelijktijdig met een stijging van de APCsr waarden en een verlaging van protein S. De hormoonspiegels gemeten in de vroege zwangerschap kwamen overeen met die tijdens hyperstimulatie en luteaal support, maar de effecten op protein S en APC resistentie waren beduidend sterker. Dit duidt erop dat tijdens zwangerschap nog andere faktoren bijdragen aan de ontstane APC resistentie.

Gedurende lange tijd werd verondersteld dat estrogeen in belangrijke mate verantwoordelijk is voor het ontstaan van veneuze trombose (geassocieerd met de pil). Het feit dat tweede en derde generatie pillen hetzelfde synthetische estrogeen in dezelfde hoeveelheid (30µg ethinyl estradiol) bevatten, is echter een aanwijzing dat de progestageen-component tenminste deels verantwoordelijk is voor het verhoogde tromboserisico tijdens derde generatie pilgebruik. Hoofdstuk 7 schetst de resultaten

van een studie waarin de effecten van de progestagenen, desogestrel en levonorgestrel vergeleken zijn op stollingsparameters in vrouwen zonder en met de FV_{Leiden} mutatie (hoofdstuk 7). In zowel draagsters als niet-draagsters van de FV_{Leiden} mutatie had de desogestrel bevattende combinatiepil een duidelijker effect op het antistollingsysteem dan de levonorgestrel bevattende pil. De effecten van het desogestrel bevattende combinatiepreparaat waren groter in draagsters van de FV_{Leiden} mutatie dan in niet-draagsters. Echter de belangwekkendste bevinding was dat tijdens het gebruik van preparaten met alleen maar progestageen, effecten gevonden werden die tegengesteld waren aan de effecten waargenomen bij de combinatiepreparaten. Het lijkt er dus sterk op dat de progestagen-component in het combinatiepreparaat de (protrombotische) werking van de estrogeen-component tegengaat. En aangezien het effect van levonorgestrel alleen in het algemeen groter was dan het effect van desogestrel alleen, kunnen deze waarnemingen de sterkere protrombotische effecten van de derde generatie (desogestrel-bevattende) pil ten opzichte van de tweede generatie (levonorgestrel-bevattende) pil mogelijk verklaren.

Kunnen veranderingen in het niveau van stolfaktoren piltrombose verklaren?

Wij hebben in al onze studies (cross-sectionele, cross-over en longitudinale opzet) laten zien dat het gebruik van combinatiepreparaten geassocieerd is met een verminderde werking van het antistollende protein C systeem, weerspiegeld in een verhoogde APC resistentie. Bovendien waren de effecten op de hemostase duidelijker bij gebruik van desogestrel bevattende pillen dan bij gebruik van levonorgestrel bevattende pillen. Onze en andere studies hebben laten zien dat het gebruik van de pil de niveaus van stollingsparameters op een belangrijke manier beïnvloedt en dat de verschillen in risico voor veneuze trombose tussen tweede en derde generatie pilgebruik hierdoor, tenminste deels, verklaard kunnen worden. Tevens hebben we laten zien dat personen met de FV_{Leiden} mutatie (die reeds een verslechterde remming van de stolling hebben) resistenter tegen APC worden tijdens pilgebruik (zeker in tijdens gebruik van desogestrel bevattende preparaten), wat het hoge risico voor veneuze trombose in FV_{Leiden} draagsters die de pil gebruiken kan verklaren.

Omdat de gerapporteerde veranderingen tijdens pilgebruik bij de meeste vrouwen voor een groot gedeelte binnen het normale gebied blijven, is de relevantie van deze veranderingen vaak ter discussie gesteld. Echter, het wordt de laatste jaren meer en meer duidelijk dat ook kleine veranderingen kunnen bijdragen aan het risico op trombose. Vaak wordt namelijk vergeten dat een kleine gemiddelde verandering van een reeds ongustige waarde net voldoende kan zijn om tot trombose te leiden. Maar ook dient er rekening mee gehouden te worden dat niet in alle personen de gemiddelde verandering optreedt. Wanneer een (uiteraard minder vaak voorkomende) grote verandering ook nog eens voorkomt bij een persoon met een ongunstige beginwaarde kan dat dit wel degelijk leiden tot trombose.